REVIEW

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The Role of CD44 in the Pathogenesis, Diagnosis, and Therapy of Gastric Cancer

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CD44 is a transmembrane glycoprotein and surface receptor for hyaluronan that is involved in the response of cells to their microenvironment. CD44 splice variants play roles in carcinogenesis, differentiation, and lymph node metastasis and are predictive of the prognosis for various carcinomas, including gastric cancer. Current data suggest that gastric tissue stem cells and gastric cancer stem cells both express the splice variant, CD44v9. Overall, the data regarding the alterations that occur in CD44 and its splice variants in response to acute and chronic infection with *Helicobacter pylori* are scant and poorly elucidated in terms of possible changes in expression that occur in gastric cancer precursor lesions, such as chronic atrophic gastritis, pyloric metaplasia and intestinal metaplasia. In this study, we discuss the available data and suggest which new data would likely be useful in clinical practice. We also discuss the potential for CD44-targeted therapeutic strategies in gastric cancer. CD44 and its splice variants are positively associated with the initiation and progression of gastric cancer and may also play important roles in diagnosis, therapy and prognosis. CD44 research has been active but fragmented, and it may offer new therapeutic approaches to gastric cancer. **(Gut Liver 2011;5:397-405)**

Key Words: *Helicobacter pylori*; Gastric cancer; CD44; Biomarkers; Chemotherapy

INTRODUCTION

Gastric cancer is the one of the leading causes of cancer related deaths worldwide. Although the incidence of gastric cancer has been decreasing, it still represents roughly 2% of all new cancer cases yearly in the United States.[1-3] Approximately 90% of gastric cancers are adenocarcinomas which are divided into 2 types based on the degree of differentiation: the more common well differentiated intestinal-type and the less common poorly differentiated or diffuse-type.[4,5] The intestinal-type of gastric cancer has long been known to be tightly associated with atrophic gastritis and gastric atrophy.[6,7] The majority of gastric cancers are end products of an inflammatory cascade that progresses from superficial nonatrophic gastritis to gastric atrophy and is associated with the development of metaplastic epithelia, including the pyloric-type also known as spasmolytic polypeptide expressing, and the intestinal type, through intraepithelial neoplasia (also known as dysplasia) to invasive cancer.[5] This cascade is caused by infection with the bacterial pathogen *Helicobacter pylori* which is a necessary but not sufficient cause of gastric cancer.

Gastric cancer is not an inevitable outcome of a *H. pylori* infection, for example, the incidence of gastric cancer often varies greatly between geographic regions despite their having an equally high prevalence of *H. pylori* infection. The pathogenesis of gastric cancer is complex and on a macro level is the result of interactions between bacteria, the host, and the environment. Factors associated with a more robust inflammatory response such as an infecting strain containing the *cag* pathogenicity island, or polymorphisms in host genes governing the response to inflammation, both increase the risk of gastric cancer for an individual patient. However, the most prominent factors separating high and low risk populations are environmental and include diets high in salt, a low intake of fruits and vegetables, and smoking.

Risk can also be stratified based on the severity and extent of atrophic gastritis which can be assessed endoscopically (e.g., Takemoto-Kimura endoscopic classification), histologically (e.g.,

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OLGA staging system), or biochemically based on serum pepsinogen assays.[7-9] Current data support the notion that gastric cancer related to *H. pylori* can largely be prevented if the infection is eradicated in the pre-atrophic stage. However, after the development of atrophic gastritis, *H. pylori* eradication can only reduce the risk of cancer but not completely prevent it.[10-13]

Overall, the prognosis of gastric cancer is poor with 5-year survivals below 24%.[1] However, endoscopic or surgical therapy of intraepithelial neoplasia or of early gastric cancers can result in cure or long survival such that surveillance for detection and removal of intraepithelial neoplasia and early gastric cancers are the main strategies to reduce the death rate from gastric cancer among high risk populations. Unfortunately, by the time stomach cancer causes symptoms, it is typically at an advanced stage with poor 5-year survival rates despite modern multimodal treatment strategies. Gastric cancer has also proven to not be particularly sensitive to current chemotherapy agents such that most therapy is palliative designed to reduce tumor size, relieve symptoms and increase survival time. Adjuvant chemotherapy to gastric cancer after surgery can modestly decrease distant recurrence.[14] Novel biomarker to predict patient outcome and new therapies to achieve better tumor response are needed. CD44 may play a role in this quest.

CD44 AND GASTRIC CANCER

CD44 is a principal cell surface receptor for hyaluronic acid, a major component of extracellular matrices. Originally described as an antigen on red blood cells and platelets, CD44 was subsequently identified as a lymphocyte homing receptor.[15-17] CD44 binds to hyaluronan and plays an important role in communication of cell-matrix interactions into the cell via "outside-in signaling." CD44 is a transmembrane glycoprotein and along with the selectins, the integrins and the cadherins are cell adhesion molecules. CD44 has been reported to play important roles in adherence to the extracellular matrices, motility, matrix degradation, proliferation and cell survival.[18-20] Cell adhesion molecules control cell behavior by mediating contact between cells or between cells and the extracellular matrix which are essential for maintaining tissue integrity. These same functions are involved in pathological functions including tumor progression and metastasis which often involve dysregulation of cell adhesion molecules (i.e., loss of E-cadherin).

CD44 is a family of glycoproteins encoded by a gene located on the short arm of human chromosome 11 (NG_008937).[21] The predominant form in epithelial cells is CD44s (standard) which consists of a link protein-homologous extracellular domains (exons 1-5 and 16), a transmembrane domain (exon 18) and a cytoplasmic domain (exon 20). CD44 isoforms arise by the alternative splicing of exons 6-15 and are designated as CD44v (variant). In theory, alternative splicing could allow more than 100 different CD44 variants all of which are of increased size compared to CD44s. CD44s is widely distributed within the body whereas CD44 variants have a much more restricted distribution and are typically expressed on epithelial cells in a tissue specific pattern. Different CD44 isoforms may have different, often additional, functions compared to CD44s.

CD44 IN GASTRIC TISSUE, NORMAL, INFLAMED, ATROPHIC, METAPLASIA, DYSPLASIA, CANCER, AND THE RELATION TO DIFFERENT VARIANTS

Data regarding CD44 expression in gastric tissue are confusing in part because different investigators have used different techniques and approaches to investigate whether CD44 and/or its splice variants are present in normal gastric tissue or are associated with various gastric pathologies. The data are further complicated by the fact many changes in gastric histology occur as a consequence of infection with *H. pylori* and these have not been systematically evaluated in terms of CD44 isoform expression. Normal gastric tissue composed of a complex mixture of cells divided into two major regions, the nonacid secreting antrum and the acid-secreting gastric corpus. Normal gastric mucosa is essentially devoid of inflammatory cells. *H. pylori* infection results in a robust inflammatory response consisting of both polymorphonuclear and mononuclear inflammatory cells as well as with progressive tissue damage and remodeling including the transformation to metaplastic epithelia. Both the inflammatory cell components and each of the different epithelial cell components could theoretically express CD44s or one or more isoforms. In addition, published results with "normal" gastric mucosa have generally not differentiated *H. pylori* infected from uninfected mucosa and authors have even gone so far as to use commercial gastric RNA as their reference material for normal stomach.[22]

The primary techniques for investigating the presence and degree of CD44 expression have been immunocytochemistry, fluorescence cell sorting, and reverse transcript polymerase chain reactions (RT-PCR). Despite the availability of polyclonal and monoclonal antibodies to CD44s and the various isoforms, most studies have used broadly reactive antibodies which therefore provide limited information regarding whether the results refer to CD44s or one or more CD44v's. Whenever possible, we will attempt to provide results in terms of CD44s and CD44 splice variants. When CD44s and CD44 isoforms were not differentiated, we will refer to the results as CD44x.

CD44 IN NORMAL STOMACH AND *H. PYLORI* INFECTION

Several studies have used immunological or RT-PCR to examine CD44 expression in "normal" stomach (Table 1).[22-26] Normal gastric epithelium strongly expresses CD44s with weak or absent expression of the various isoforms. As noted previously, normal may often include *H. pylori* infected mucosa. One study

Table 1. CD44 Expression in the Normal Gastric Epithelium

	CD44s	CD44v6	CD44v7	CD44v8	CD44v9	CD44V9*I
Sneath, *et al*.[23, †]	+++	+	+	+	ND	+++
Higashikawa, *et al*.[24, †]	+++	+	+	+	ND	ND
da Cunha, *et al*.[22, †]	ND	+	ND	ND	ND	ND
Fan, *et al*.[25,‡]	+++	+	ND	ND	+[‡]	ND
da Cunha, *et al*.[22,§]	+++	ND	ND	ND	ND	ND
Reihani-Sabet, *et al*.[26,§]	ND	ND	ND	Pos	Pos	ND

ND, not done.
*v9+v8 and or v7; [†] Immunocytochemistry, [‡] Only expressed in *H. pylori* infectio; [§]RT-PCR.

that specifically examined *H. pylori* infected and uninfected stomachs[26] used nested RT-PCR to identify CD44 splice variants V2 to V10 in *H. pylori* infected and uninfected gastric mucosal biopsies and in relation to the presence of inflammation. However 71% of the *H. pylori*-negative biopsies showed chronic inflammation which was not further described and they may have been infected or have previously been infected. In addition, immunocytochemistry was not done to confirm protein expression or to localize the expression to a specific region of the stomach or cell type. With those caveats, they reported expression of CD44v8, v9, and v10 in approximately 40% of those with *H. pylori* gastritis, 15% to 20% of those with *H. pylori*-negative noninflamed gastric mucosa, and 18% to 24% of those with *H. pylori*-negative biopsies with chronic gastritis. RT-PCR for CD44v's 2 to v7 were negative. They concluded that CD44v's 8 to 10 were expressed in both normal and *H. pylori* infected gastric mucosa and that the presence of inflammation did not significantly change expression. Fan *et al*.[25] used CD44s, CD44v6, and CD44v9 antibodies to study gastric epithelial cells and intraepithelial lymphocytes in *H. pylori* infected and uninfected individuals. Normal gastric epithelial cells and intraepithelial lymphocytes both expressed CD44s and CD44v6. Cd44v9 was not expressed on the gastric epithelium of *H. pylori* negative individual but was present in *H. pylori* infection (Table 1). Expression of CD44s on gastric epithelial cells but not on intraepithelial lymphocytes was increased in the presence of *H. pylori* infection.[25] Yasui *et al*.[27] studied presumably *H. pylori* infected gastric mucosa with CD44v9 specific antibodies and reported expression of CD44v9 in the basolateral membranes of pyloric gland cells, in gastric adenomas, and in gastric carcinomas. *H. pylori* infection was also been reported to upgrade CD44x expression in the gastric epithelial cell line, AGS cells, but as this cell line was derived from a well differentiated gastric cancer and its response visa-a-via normal mucosa is unclear.[28]

H. pylori infection has also been reported to increase the proportion with CD44v6 (i.e., in 63% of those with *H. pylori* infection vs 45% in those without).[29] In presumably *H. pylori* infected gastric mucosa, da Cunha *et al*.[22] reported very faint expression of CD44v6 localized to cells of the neck zone of the gastric glands and focally in deep glands of the gastric antrum.

CD44 IN GASTRIC CANCER

A significant stepwise increase in CD44v6 immunohistochemical expression has been reported from normal gastric mucosal biopsies (rare and weak), intestinal metaplasia, gastric mucosa adjacent to but uninvolved with gastric carcinomas, intestinal metaplasia adjacent to the tumor, and the tumor itself causing the authors to suggest that CD44v6 expression is a late-stage phenomenon in the progression from normal mucosa to gastric carcinoma.[30] da Cunha *et al*.[22] using fluorescent immunocytochemistry confirmed that CD44v6 was rarely expressed in normal gastric mucosa but was increasingly expressed in gastric hyperplastic polyps, complete and incomplete intestinal metaplasia, and was overexpressed in low- and high-grade dysplasia and malignant lesions. They concluded CD44v6 is expressed *de novo* in premalignant, as well as in sporadic and hereditary malignant lesions of the stomach and suggested that the presence of CD44v6 was potentially a biomarker signaling early transformation of the gastric mucosa.

The proportion of cases of diffuse vs intestinal type gastric carcinoma expressing CD44v5 and/or CD44v6 varies among studies and no definitive statement can be made at this time.[22,27,30-43] Comprehensive studies taking into account different isoforms, tumor histology, and prognosis will be needed to resolve this confusion. In brief, the expression of CD44v6 seems to correlate with the degree of tumor differentiation.[40] CD44v5 expression has been related to advanced grade, lymph node metastases.[44] Most signet ring carcinomas seem to express CD44v5 whether evaluated in terms of RNA or protein expression whereas intestinal-type carcinomas often express both CD44v5 and CD44v6.[42,44] Finally, a positive correlation has been described between total CD44 and CD44v9 expression in primary tumors and tumor recurrence and mortality.[45]

Intestinal metaplasia, a precancerous lesion, expresses CD44v5 and CD44v6 which is similar to the pattern in intestinal-type tumors.[42] Heider *et al*.[42] suggested that the difference in CD44 variant expression between diffuse-type and intestinal-type tumors by RT-PCR and immunochemistry would allow the two types to be discriminated on the basis of these molecular markers and that the expression of CD44v6 within precancer-

ous tissue allowed it to be easily and rapidly distinguished from normal gastric mucosa.

CD44 AND THE METASTATIC PROCESS

Günthert *et al*.[46] provided the first example of cell adhesion molecules playing a role in the metastatic process when they transfected plasmids expressing CD44s or CD44 isoforms into nonmetastatic rat pancreatic carcinoma cells and showed a significant relationship between CD44v6 expression and lymph node metastasis, lymphatic invasion, depth of invasion and tumor stage. Their experiments prompted a host of studies investigating the possible role of CD44v6 in human cancer. In gastric cancer CD44v6 expression has also been implicated in the development of lymph node metastasis, hematogenous metastasis, invasion and the pathological grade of the tumor (Tables 2 and 3).[31,33-35,38-41,47] In one study of submucosal gastric carcinoma CD44v6 was found to be the only indicator of lymph node metastasis.[39] In gastric micropapillary carcinoma CD44v6 expression was associated with a higher T classification, lymph node metastasis, and lymphovascular invasion. Detection of CD44v6 mRNA in blood and bone marrow has also been reported to be sensitive and specific marker of micrometastasis.[38] In contrast, CD44v5 has been reported to be preferentially expressed in poorly differentiated type gastric cancer and in metastatic lymph nodes.[36,37]

GASTRIC CANCER STEM CELLS CD44 VARIANTS

Cancer stem cells drive tumorigenesis and also give rise to the large population of differentiated progeny that make up the bulk of the tumor (i.e., they possess the ability to initiate tumor growth and sustain tumor self-renewal).[48,49] Cancer stem cells make up a small fraction of cell in leukemia and solid tumors such as breast,[50] pancreas,[51] head and neck squamous cell carcinoma (HNSCC),[52] colon,[53] and prostate cancers.[54] In 2009, Takaishi *et al*.[55] used three gastric cancer cell lines with sizeable subpopulations of CD44 positive cells to identify gastric stem cells. They injected cells from these cell lines into the stomach and skin of severe combined immunodeficient (SCID) mice and found that only the CD44 positive cells showed tumorigenic ability and the stem cell properties of self renewal and differentiation. SCID mice given CD44 positive cells developed tumors within 8 to 12 weeks and CD44 knockdown by short hairpin

Table 2. Expression of CD44 or CD44 Splice Variants in Gastric Cancer

Study	CD44 or CD44v	Specimen	Geographic area	CD44 or CD44v positive cases/ Normal tissue cases (%)	CD44 or CD44v positive cases/ Gastric adenocarcinoma cases (%)	CD44 or CD44v positive cases in different pathologic types (%)
Ghaffarzadehgan *et al*. (2008)[33]	CD44	Gastric mucosa	Iran	0/100 (0)	65/100 (65)	IT 56/78 (72), DT 9/22 (41)
Kim *et al*. (2005)[47]	CD44	Gastric mucosa	Korea	NA	142/729 (19)	Cardia 46/165 (28) noncardia 96/564 (17.0)
Liu *et al*. (2005)[40]	CD44s	Gastric mucosa	China	3/22 (13.6)	19/40 (47.5)	
	CD44v6	Gastric mucosa	China	0/22 (0)	25/40 (62.5)	
Wang *et al*. (2006)[38]	CD44v6	Blood	China	0/14 (0)	39/46 (84.8)	IT 9/14 (64.3), DT 30/32 (93.8)
	CD44v6	Bone marrow	China	NA	40/46 (87.0)	IT 10/14 (71.4), DT 31/32 (96.9)

IT, intestinal-type cancer; DT, diffuse-type cancer.

Table 3. Expression of CD44v6 and the Clinicopathologic Characteristics of Gastric Cancer

Study	Cases of gastric cancer	Conclusion
Xin *et al*. (2001)[34]	55	CD44v6 expression was positively correlated with advanced stage. Strong positivity was only detected in those with metastases. Patients with CD44v6 positive tumors revealed a lower 3- and 5-year survival rate.
Joo *et al*. (2003)[41]	99	CD44v6 showed significant relationships with lymphovascular invasion and TNM stage.
Liu *et al*. (2005)[40]	62	The expression of CD44v6 was significantly associated with the lymph node metastasis, invasion and pathological grade of the tumor.
Chen *et al*. (2004)[35]	43	The CD44v6 protein expression was significantly related to serosal infiltration, lymph node metastasis, and TNM stage of disease.
Yamaguchi *et al*. (2002)[31]	201	CD44v6(+) cancers were more frequently associated with hematogenous metastasis, the prognosis was significantly poorer in patients with CD44v6(+) tumors than in those with CD44v6(-) tumors.
Okayama *et al*. (2009)[39]	135	CD44v6 was significantly associated with lymph node status.

RNA resulted in a decrease in tumorigenic ability.[55] Furthermore, CD44 positive gastric cell showed significant resistance to chemotherapy or radiotherapy. Authors used fluorescent cell sorting to separate CD44 positive and negative cells. Their antibody broadly reacted with all CD44 isoforms such that they were unable to address whether the effect was CD44v-specific.[55]

CD44v9 appears to be the current most likely candidate stem cell marker. CD44v9 expression in the gastric mucosa was previously shown to be positively correlated with proliferative activity as assessed by Ki-67 expression[27] and, in addition, CD44v9 was found to be co-expressed with Ki-67. CD44v6 was also reported to be associated with expression of p53 but the expression was in different cells and in colorectal carcinoma, variant CD44 expression was found to precede p53 gene mutation in the adenoma-carcinoma sequence.[56] One of the features of cancer stem cells is its resistance to chemotherapy, radiotherapy and reactive oxygen stress. Recently, it has been shown that CD44v9 regulates redox status in gastric cancer cells which further links CD44v9 and gastric cancer stem cells.[57] Of interest, tissue stem cells are characteristically slow-cycling cells that are also Ki-67 negative. Recently, Ishimoto *et al*.[58] showed that what appeared to be gastric cancer stem cells in mice expressed CD44v9 (the antibody was to CD44V8-V10). Slow cycling and presumably tissue stem cells found in a normal squamocolumnar gland were also CD44v9 positive but were Ki-67 negative suggesting that tissue stem cells and gastric cancer stem cells may be able to be differentiated in terms of cycling (e.g., Ki-67 expression or BrdU uptake). Whether differences exist in the associated CD44v9's (e.g., amount of glycosylation, inclusion of additional genetic material such as intron 9, etc.) is unknown (see below).

CD44 AND RESPONSE TO CHEMOTHERAPY

Resistance of cancer to chemotherapy and radiotherapy, and recurrence of cancer is one attribute of cancer stem cells. CD44 acts as a common downstream effector of RAS, and is considered a stem marker responsible for tumor progression and resistance to therapy.[59] Oxidative stress occurs when production of reactive oxygen species (ROS) exceeds the capacity of the cellular defense system consisting of redox enzymes and other antioxidant molecules. Like normal tissue stem cells, subsets of cancer stem cells in some tumors harbor low levels of ROS and manifest enhanced mechanisms for protection against reactive oxygen species-mediated damage.[60] Recent studies suggest that CD44-mediated reactive oxygen species resistance is independent of antioxidant gene expression and is the result of an interaction between CD44v9 and the cystine transporter subunit xCT which controls the intracellular levels of glutathione.[57] Cancer stem cells and normal stem cells possess an enhanced reactive oxygen species defense system[60] suggesting that CD44v9 may be important in stem cells of various tumor types.[57] The presence of CD44v9 in cancer stem cells also provides a rationale for CD44v-targeted therapy to impair reactive oxygen species defense in cancer cells in order to sensitize them to current treatments.

CD44 GENE POLYMORPHISMS AS A MARKER FOR OUTCOME

The mortality associated with gastric carcinoma is almost entirely caused by metastatic disease such that the prognostic assessment relies mainly on TNM staging. However, wide individual variability in prognosis is observed even within the same stage. Better prediction of metastatic potential of the primary tumor would assist in the management of patients with gastric carcinoma. Expression of CD44v9 has been correlated with the expression of Ki-67, development and the progression of the gastric carcinoma.[27] Recently, Winder *et al*.[61] reported that germline polymorphism in the CD44 gene, at least one G allele (GG; AG) at the CD44 +4883G>A gene locus (rs187116) associated with clinical outcome in patients with localized gastric adenocarcinoma. They found that patients having at least one G allele of CD44 rs187116 remained significantly associated with time to recurrence and overall survival and that patients harboring CD44 T-A haplotype were at the lowest risk of developing tumor recurrence and death. These results suggest that assessment of CD44 polymorphisms may assist in identifying patients with localized gastric cancer who are at high risk for tumor recurrence.

ROLE OF CD44 IN CANCER THERAPY

Based on data from transgenic mice and humans, it appears that CD44v9 is expressed on both gastric tissue stem cells and gastric cancer stem cells. However, gastric cancer stem cells also co-express Ki-67 whereas tissue stem cells cycle slowly and frequently do not. Prior studies showed that CD44v9 was also co-expressed with Ki-67 in nonmetaplastic gastric mucosa not associated with gastric cancer. It seems likely that cells in "normal" gastric glands that co-express CD44v9 and Ki-67 may be daughter cells produced from gastric tissue stem cells and that CD44v9 is subsequently lost as the cells further differentiate into normal gastric epithelium. CD44v6 is expressed on differentiated gastric cancer cells. The fact that different CD44 isotypes are expressed on gastric cancer stem cells and on differentiated gastric cancer glandular cells suggests a possible role for isoform specific anti-CD44 therapy.

Methods to silence the CD44 gene include using a vector such as a lentiviral or pSico vector carrying a short hairpin RNA (shRNA) against CD44[55] or transfecting CD44 small interfering RNA (siRNA) in order to knockdown CD44.[62,63] However, currently these methods are primarily applicable to *in vitro* studies and their applicability to gastric cancer remains unclear. How-

ever, there has been considerable work in other systems. For example, in an animal study, Misra *et al*.[64] intravenously injected pSico-CD44v6 shRNA plus a intestine-specific pFabpl promoter-driven-Cre-recombinase plasmid packaged in transferring-coated nanoparticles into a mouse model of intestinal adenoma and silenced CD44v6-v9mRNA expression, inhibited protein expression of CD44v6 variants (v6-9) and reduced tumor number with only a limited effect on CD44s-normal tissue.

CD44v interactions with hyaluronan are involved in the metastatic cascade. A number of methods to change CD44-interactions have been developed including the use of small hyaluronan oligosaccharides, use of soluble CD44 to act as competitive decoys for CD44, use of blocking antibody against the hyaluronan binding site, and finally inhibition of the post translational expression of CD44v with siRNA (reviewed in reference 65) CD44 can internalize hyaluronan and thus is a target receptor for hyaluronan-conjugated drugs, nanocarrier delivery systems, or for anti-CD44 antibodies linked to radioactive isotope or chemotherapeutic agents.[65] For example, an abioconjugate of hyaluronic acid with SN-38 chemotherapy has been used to target CD44 as the receptor for hyaluronan. This approach showed higher inhibitory activity on a gastric cancer cell line than exerted by unconjugated SN-38.[66] Hyaluronan-conjugated with cisplatin-loaded microparticles have been injected into peritoneum of mice with ovarian cancer producing increased uptake of cisplatin in the CD44 expressing cancer cells resulting in inhibition of tumor growth.[67] Liu *et al*.[68] used a liposomal nanovector to deliver miR34a, (microRNA-34a), a key negative regulator of CD44 in prostate cancer cells, in a mouse model of prostate cancer to inhibit tumor growth and metastasis and prolong survival. Bivatuzumab (BIWA4) a humanized monoclonal antibody against CD44v6 linked to radioisotopes for use in radiotherapy or to an antineoplastic drug for chemotherapy has been used in phase I trials in head and neck cancer showing high tumor uptake along with sparing of normal tissue.[69-72] Finally, an anti-CD44v6 antibody linked to the cytotoxic agent mertansine was used to stabilized the disease in patients with breast cancer refractory to conventional chemotherapy.[73]

A challenge remains on how target antibodies to the specific CD44v uniquely expressed on the targeted cancer. As noted above, future research needs to be directed to identifying unique features within the CD44 gene and in the CD44 variants expressed so as to provide specificity to the regimen and allowed truly tailored treatment strategies. RT-PCR amplification and hybridization have shown that tumor cells exhibit a complex pattern of variant CD44 transcripts and that different CD44v patterns occur in different primary gastric tumors and in the lymph node metastasis derived from those tumors.[42] Studies will be needed to identify the relation between the target(s) of CD44 antibodies and the data derived from studies using RT-PCR.

FUTURE RESEARCH

Understanding the roles of CD44 and its isoforms in the pathogenesis and treatment of gastric cancer would be enhanced by better understanding of the role and expression of CD44s and CD44 splice variants in *H. pylori*-infection, especially in relation to the various *H. pylori*-associated gastroduodenal pathologies. Not only is the current data scanty in terms of which isoform or isoforms are expressed but extends to the relation of their expression in different regions of the stomach and in the different immunological and epithelial cell types present. Information needed includes which isoforms are present, are the co-expressed on the same cell, what other important factors are co-expressed (e.g., Ki-67), and whether there are differences between what appears to be same isoform present on gastric tissue and gastric cancer stem cells (e.g., different glycosylation or different carbohydrate antigens, or genes such as intron 9, expressed). The reagents needed to obtain these data are currently available as are the tissue samples making collection of such data primarily a matter of priority rather than requiring overcoming technical problems.

CONFLICTS OF INTEREST

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Dr. Li, Jang, and Cen have nothing to declare.

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